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Original Article

Prevalence of *Helicobacter pylori* Infection in Japan: Relation to Educational Levels and Hygienic Conditions

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Abstract

Epidemiological characteristics and multiple risk factors of *Helicobacter pylori* infection in Japan were studied by both descriptive and analytical studies. We attempted to find out the association between some hygienic factors and *Helicobacter pylori* infection in Japan. Effects of lifestyle factors in later life time on the infection was also considered. Whole employees of a Japanese company were screened by Enzyme-linked Immunosorbent Assay methods (ELISA). Healthy (no current gastroenterological diseases) subjects' childhood hygienic conditions and later life time lifestyles were surveyed by a questionnaire. A significant age-dependent prevalence of *Helicobacter pylori* infection was found in the studied subjects. Experience of well-water drinking and no flush-toilet using in childhood as well as low educational level were shown significantly related to the infection. On the contrary, later life time lifestyle did not seem to affect the infected condition. Our study confirmed that early childhood hygienic condition had important effects on *Helicobactor pylori* infection.

Key words: *Helicobacter pylori*, educational level, hygienic condition, lifestyle

Introduction

Helicobacter pylori (*H. pylori*), a curved microaerophilic Gram-negative rod, which was first isolated by Warren and Marshall in 1982[1,2], colonized beneath the human mucous layer. During a decade's intensive research, it has been associated with the pathogenesis of gastritis, gastric and duodenal ulcer and gastric cancer[3]. Its eradication, not only results in curing and preventing recurrence of gastric ulcer, but also benefits patients with MALT lymphoma[4].

Transmission mode and risk factors for *H. pylori* infection have not yet been clearly understood, though it is believed that it may be transmitted by person to person, fecal-oral, or via a common source[5-8]. There must be multiple etiologic factors, including both genetic and environmental[9]. Most people were infected in their early childhood[10,11]. Currently the infection has been associated with low socioeconomic status, such as lack of hot water in the home, overcrowding and sharing of beds[12]. The prevalence rates are usually lower in developed countries than in developing nations[13,14]. However, even in the developed countries, the prevalence of *H. pylori* has been inversely related to socioeconomic status in the studies in Korea and United States. Therefore, we tried to find out the possible hygienic conditions and later time lifestyle factors that affected the infection in Japan.

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Methods

Study population

Subjects in this study were employees (male 472, female 126) working in a manufacturing plant in Japan. In order to restrict the age group, we excluded those who were under 20 years old (4 males) or above 59 (3 males) from the study. Eleven persons with current gastroenteric diseases were also excluded. Five hundred and forty-five (94% of total emplyees) participants were enrolled in the study and completed a self-administrated questionnaire at an annual health examination in July 1996. All subjects participated in the study with informed consent. There were 294 (68.9%) non-clerical workers and 127 (29.7%) clerical workers among male subjects, and 60 (50.8%) non-clerical workers and 55 (46.6%) clerical workers among females subjects. Other characteristics of the subjects were shown in Table 1. Peripheral blood was drawn from each subject and utilized for serological testing of *H. pylori* antibody.

Serological testing

Serum samples were assessed for *H. pylori* antibody IgG by using commercially available high-molecular-weight cell-associated protein (HM-CAP) *H. pylori* immunoassay

(Determiner *H. pylori* Antibody, Enteric Products Inc.), according to the kit's protocol. Briefly, 5 μL of sera was diluted in 500 μL buffer. One hundred microliters of diluted solution was put into a 96-well microtiter plate, in which it was incubated at room temperature for 20 minutes, and then washed for three times. Next, enzyme linked antibody was added and further incubated for 20 minutes. The substrate liquid was then added and incubated for 10 minutes. Finally, the reaction was stopped by stop liquid. Each plate had one set of high positive, low positive and negative control. The optical density values were detected by a plate reader (Emax precision micro plate reader, Molecular Devices Inc.) at 450 nm wavelength, and then converted to ELISA Value (EV) set by Enteric Products Incorporated (EPI). Subjects whose EV values were equal to or above the cut-off value of 1.4 were viewed as *H. pylori* infection positive subjects, and those below 1.4 were defined as *H. pylori* infection negative subjects. We had tested the sensitivity and specificity of the kit among 96 positive and 27 negative Japanese subjects examined by both Rapid Urease Test and Histological Test. It was found that our cut-off value (1.4) had a higher sensitivity (90.6% vs. 82.3%) than that of the kit's recommendation value (1.8), whereas their specificities were the same (92.6%).

Questionnaire

A self-administrated questionnaire was designed to collect the demographic data such as, age, sex, weight, height and educational level. Early childhood hygienic conditions (experience of well-water drinking and flush toilet using before 12 years old) was obtained. Later time lifestyle factors including smoking, drinking and stress were also investigated. The condition of cigarette smoking was asked as whether they were non-smoker, current smoker or ex-smoker, along with length of their smoking history. The alcohol consumption habit was asked as self-conscious stress, which was divided into three groups as excessive, moderate and less stress.

Statistical analysis

The ELISA positives were defined as *H. pylori* infection positives after serum analysis. Epidemiological and serologic data were analyzed by SPSS statistical package (SPSS Inc., Chicago, IL). *Chi* square test was used to analyze the association between all observed variables and *H. pylori* infection. The difference of educational level and hygienic conditions between the 20-39 and 40-59 age groups was also analyzed by χ^2 test. As to those potential risk factors, their relationships were analyzed by multivariate logistic regression models. In all tests p values under 0.05 were considered significant.

Table 1 Univariate analysis of variables for *Helicobacter pylori* infection

Variables	Anti-*H. pylori* IgG		
	Positive (n=216)	Negative (n=329)	p value[a]
Gender			
Women	45 (38.1)	73 (61.9)	-
Men	171 (40.0)	256 (60.0)	0.707
Age group (years)			
20-29	25 (17.9)	115 (82.1)	-
30-39	37 (31.1)	82 (68.9)	0.013
40-49	82 (49.4)	84 (50.6)	0.000
50-59	72 (60.0)	48 (40.0)	0.000
Education			
College graduate	66 (34.2)	127 (65.8)	-
Senior high school graduate	106 (39.6)	162 (60.4)	0.241
Junior high school graduate	43 (54.4)	36 (45.6)	0.002
Using flush toilet[b]			
Occasional & regular	43 (26.9)	117 (73.1)	-
No experience	173 (44.9)	212 (55.1)	0.000
Drinking well water[b]			
No experience	133 (35.0)	247 (65.0)	-
Occasional & regular	81 (50.6)	79 (49.4)	0.000
Cigarette smoking			
Non- smoker	90 (39.6)	137 (60.4)	-
Current & ex-smoker	126 (39.7)	191 (60.3)	0.981
Alcohol consumption			
None & occasional[c] drinker	89 (34.5)	169 (65.5)	-
Habitual drinker	127 (44.3)	160 (55.7)	0.020
Mental stress			
Less	13 (26.0)	37 (74.0)	-
Moderate & excessive	203 (41.0)	292 (59.0)	0.039

Values shown are numbers of subjects (percentage).
a: By χ^2 test
b: Experienced before 12 years old.
c: Person who drinks less than one time per week.

Results

General characteristics

As shown in Figure 1, the prevalence of *H. pylori* infection had significant association with age groups when analyzed by logistic regression model (OR=1.97, 95%CI=1.65-2.34 in every ten years) in the whole study population. No significant difference in the *H. pylori* infection prevalence between male and female was observed, though there was a relative low proportion of female participants (Table 1). The extremely high prevalence in female 30-39 age group may be due to chance because of its

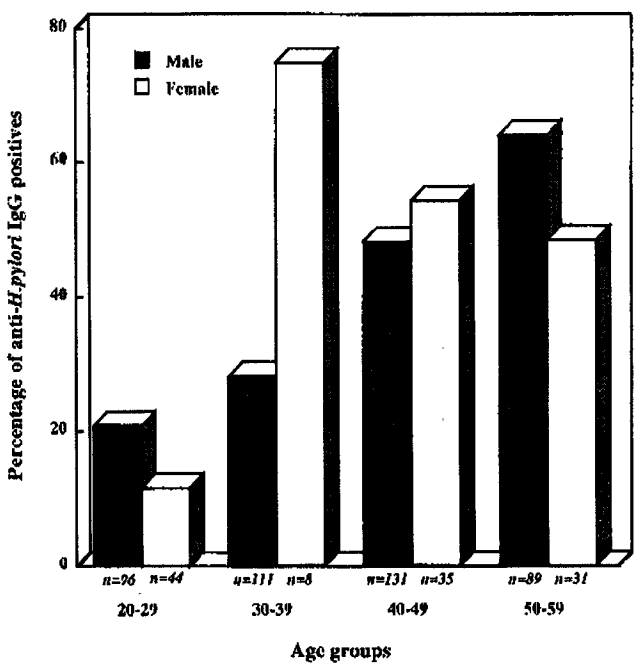

Fig. 1 The prevalence of *H. pylori* infection according to age groups.
The italic number below the bar indicates total number of subjects in each group.

Table 3 Multivariate logistic regression analysis of risk factors and their association with *Helicobacter pylori* infection

Risk factors	Model I		II		III	
	OR[a]	95%CI[a]	OR	95%CI	OR	95%CI
Education						
College graduate	1.0		1.0		1.0	
Senior high school graduate	1.20	0.80-1.74	1.15	0.76- 1.74	1.17	0.77- 1.77
Junior high school graduate	1.85	1.07-3.21	1.18	0.65- 2.15	1.20	0.65- 2.19
No flush toilet[b]	1.83	1.19-2.81	1.07	0.66- 1.73	1.12	0.69- 1.81
Drinking well waterb[c]	1.58	1.07-2.34	1.20	0.79- 1.82	1.22	0.80- 1.85
Age group (years)						
20-29			1.0		1.0	
30-39			2.04	1.12- 3.73	1.92	1.04- 3.52
40-49			4.25	2.38- 7.60	3.93	2.18- 7.10
50-59			6.20	3.16-12.14	5.60	2.81-11.15
Alcohol consumption					1.24	0.85- 1.81
Mental stress					1.98	0.98- 4.01

Model I, risk factors as early childhood hygienic condition and educational level; Model II, all risk factors in Model I plus subjects' age; Model III, all risk factors in Model II plus later time lifestyle factors.

a: OR, odds ratio; CI, confidence interval. b: Experienced before 12 years old. c: Includes occasional & regular drinker.

Table 2 Educational level and hygienic condition between 20-39 and 40-59 age groups

Risk factors	20-39 age group (n=259)		40-59 group (n=286)	
High school graduate[a]				
Senior	136	(52.5)	132	(46.2)
Junior	11	(4.2)	68	(23.8)**
No flush toilet[b]	131	(50.6)	254	(88.8)**
Drinking well waterb,[c]	41	(15.8)	119	(41.6)**

Values shown are numbers of subjects (percentage).

a: Percentage of risk factor having subjects according to its age group.

b: Experienced before 12 years old.

c: Includes occasional & regular drinker

** $p<0.01$ by χ^2 test

limited sample size.

Educational level and hygienic conditions

Educational level affected the *H. pylori* infection prevalence (Table 1). When compared with college graduates (34.2%), high school graduates had a higher seropositivity rate, in which senior high school graduate was 39.6%, and junior high school graduate was (54.4%, $p=0.002$). As to the early childhood hygienic conditions, we found that those who did not have flush toilet using experience had a higher *H. pylori* infection prevalence rate (44.9%, $p=0.000$) than that of occasional and regular users. A similar difference was also observed between the well-water drinkers (50.6%, $p=0.000$) and non-drinker.

We found significant differences between 20-39 and 40-59 age groups in both the educational levels and the early childhood hygienic conditions (Table 2). The proportion of the subjects who had low educational levels and poor hygienic conditions was significantly higher in the 40-59 age group ($p<0.01$)

Risk factors and H. pylori infection

In order to find out the association between *H. pylori* infection and independent risk factors, we made use of three different multivariate logistic regression model to analyze them (Table 3). In model I, after adjusted for gender, we evaluated effects of the educational level and early childhood hygienic conditions. We found that all three risk factors were significantly related to *H. pylori* infection. High *H. pylori* prevalence rate was observed in subjects with low educational level or poor early childhood hygienic conditions. In model II, we added four age groups in the model I, and found that all other risk factors' significance disappeared, except for age groups themselves. Finally, in model III, we further added in two lifestyle factors (alcohol consumption and mental stress). These two lifestyle factors were significantly associated with *H. pylori* infection by univariate analysis (Table 1), however, their significance vanished in the multivariate model.

Discussion

Although the transmission mode of *H. pylori* remains unclear, the acquisition of the infection is known to be in early childhood[15]. It has also been associated with variable genetic and environmental factors, including socioeconomic[16] and hygienic status[12], through epidemiological investigation. Malaty and Graham reported that, in the United States, the prevalence of *H. pylori* infection in African-Americans and Hispanics is inversely related to social class during childhood[17]. They also indicated that, in Korea, the prevalence of *H. pylori* infection in children has an inverse relation to socioeconomic status despite a uniformly high prevalence in adults[16].

Japan is a country that has a comparatively steady genetic predisposition, and that its society also is not so polarized in economical status. Therefore, people usually have a relatively similar living standard and living habits. Japan has a rapid economic development during 1960s, accompanied with a great improvement in its general hygienic level. Part of that hygienic improvement was shown in this study because the percentage of people who did not have a flush toilet using experience or who drank well water in their early childhood was significantly higher in the 40-59 age group than that in the 20-39 age group. When analyzing the relationship between those two hygienic factors and *H. pylori* infection, we found that both factors were significantly related to the infection not only by univariate analysis, but also by multivariate logistic regression analysis. Therefore, it is suggested that these two factors might be possible risk factors in the acquisition of *H. pylori* infection through fecal-oral transmission. The educational level was also significantly related to *H. pylori* infection, especially when comparing the prevalence rate between college graduates and junior high school graduates. From another point of view, educational attainment may also be

considered as an indicator of childhood socioeconomic level of the subjects or their family, which means children from poorer families completed fewer years of education. However, due to the difficulty in collecting such kind of data, we are not able to clarify this point in this study. The different hygienic condition and educational levels here, can probably explain why there is a significant difference in the *H. pylori* acquisition rate between the 20-39 and 40-59 age group. This result is consistent with other reports describing *H. pylori* infection prevalence in Japan[18-21].

It is reported that most people are infected in their childhood [10,11] and they are seldom infected in their adulthood[22], which meant childhood hygiene conditions and other factors determined whether a person would be infected or not. In the present study it was shown that both hygienic conditions and educational levels were highly different between different age groups. Therefore, if we wanted to evaluate the effects of early childhood hygienic conditions and educational levels, it would be proper to compare their conditions all at their early childhood, which should not be confused by their real age. Therefore, we should not add subjects' current real age in the model I. If we included age in the model, as what could be seen in model II, the above observed associations were hidden.

However, when we analyze the relationship between *H. pylori* infection and later time lifestyle factors, we should adjust that for age, since lifestyle is a long time personal behavior. Age did have a confounding effect when studying the relationship between alcohol consumption and *H. pylori* infection by the univariate analysis, because there was a high percentage (60.6%) of 40-59 age group subjects in the habitual drinker group. Therefore, when we adjusted the association between alcohol consumption and the infection for age in the multivariate model, its significance disappeared. There is still no basic agreement on the role of alcohol consumption, because alcohol was reported to have both beneficial and adverse effects on *H. pylori* infection by its increasing mucus barrier permeability or its invocation of mucosal defense function[20,23,24]. Similar to other reports[3,16,25,26], our study cannot find any correlation between smoking and *H. pylori* infection.

In model III, the results of association between mental stress and *H. pylori* infection was marginally insignificant, though their relation was once significant by the univariate analysis. The explanation is that the size of less stress group is too small when comparing that of moderate and excessive stress group in this study. Only if we enlarge our sample size, we could possibly address the effect of stress more precisely.

The present study is limited by itself as a cross-sectional investigation. In the following study we want to enlarge the size of 20-39 age group in order to find out whether hygienic conditions or educational levels still act as a risk factor for the infection among younger generation.

In summary, the results of this study indicate that in Japan the acquired rate of *H. pylori* increased with age groups. A considerable difference of prevalence rate was observed between the 20-39 age group and 40-59 age group, which was possibly affected by their different educational levels and hygienic conditions. Gender, cigarette smoking and alcohol consumption were found to have no relation to *H. pylori* infection.

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References

1) Marshall B, Warren J. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; I : 1311-5.
2) Warren J, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; I : 1273-5.
3) Parsonnet J, Friedman GD, Vandersteen DP, et al. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991; 325 : 1127- 31.
4) Parsonnet J, Hansen S, Rodriguez L, et al. *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med 1994; 330 : 1267-71.
5) Drumm B, Perez Gi, Blaser MJ, et al. Intrafamilial clustering of *Helicobacter pylori* infection. N Engl J Med 1990; 322 : 359-63 .
6) Mitchell HM, Bohane TD, Berkowicz J, et al. Antibody to *Campylobacter pylori* in families of index children with gastrointestinal illness due to *C. pylori.* (letter). Lancet 1987; 2 : 681-2.
7) Malaty HM, Graham DY, Klein PD, et al. Transmission of *Helicobacter pylori* infection: studies in families of healthy individuals. Scand J Gastroenterol 1991; 26 : 927-32.
8) Jones DM, Eldridge J, Whorwell PJ. Antibodies to *Campylobacter pyloridies* in household contacts of infected patients. Br Med J (Clin Res Ed)1987; 294 : 615.
9) Malaty HM, Engstrand L, Pedersen NL, Graham DY. *Helicobacter pylori* infection: genetic and environmental influences. A study of twins. Ann Intern Med 1994; 120 : 982-6.
10) Kuipers EJ, Pena AS, van Kamp G, et al. Seroconversion for *Helicobacter pylori.* Lancet 1993; 342 : 328-31.
11) Mitchell HM, Li YY, Hu PJ, et al. Epidemiology of *Helicobacter pylori* in southern China: identification of early childhood as the critical period for acquisition. J Infect Dis 1992; 166 : 149-53.
12) Megraud F. Epidemiology of *Helicobacter pylori* infection. Gastroenterol Clin North Am 1993; 22 : 73-88.
13) Fennerty MB. *Helicobacter pylori.* Arch Intern Med 1994; 154 : 721- 7.
14) Graham DY, Adam E, Reddy GT, et al. Seroepidemiology of *Helicobacter pylori* infection in India: comparision of developing and developed countries. Dig Dis Sci 1991; 36 : 1084-8.
15) Goodwin C, Mendall M, Northfield T. *Helicobacter pylori* infection. Lancet 1997; 349 : 265-9.
16) Malaty HM, Kim JG, Kim SD, Graham DY. Prevalence of *Helicobacter pylori* infection in Korean children: inverse relation to socioeconomic status despite a uniformly high prevalence in adults. Am J Epidemiol 1996; 143 : 257-62.
17) Malaty HM, Graham DY. Importance of childhood

socioeconomic status on the current prevalence of *Helicobacter pylori* infection. Gut 1994; 35 : 742-5.
18) Anonymous. Epidemiology of, and risk factors for, *Helicobacter pylori* infection among 3194 asymptomatic subjects in 17 populations. The EUROGAST Study Group. Gut 1993; 34 : 1672-6.
19) Asaka M, Kimura T, Kudo M, et al. Relationship of *Helicobacter pylori* to serum pepsinogens in an asymptomatic Japanese population. Gastroenterology 1992; 102 : 760-6.
20) Tsugane S, Tei Y, Takahashi T, Watanabe S, Sugano K. Salty food intake and risk of *Helicobacter pylori* infection. Jpn J Cancer Res 1994; 85 : 474-8.
21) Shinchi K, Ishii H, Imanishi K, Kono S. Relationship of cigarette smoking , alcohol use, and dietary habits with *Helicobacter pylori* infection in Japanese men. Scand J Gastroenterol 1997; 32 : 651-5.
22) Cullen DJ, Collins BJ, Christiansen KJ, et al. When is *Helicobacter pylori* infection acquired? Gut 1993; 34 : 1681-2.
23) Hook NJ. Effect of alcohol consumption on the risk of *Helicobacter pylori* infection. Digestion 1991; 50 : 92-8
24) Battaglia G, Di MF, Pasini M, et al. *Helicobacter pylori* infection, cigarette smoking and alcohol consumption. A histological and clinical study on 286 subjects. Ital J Gastroenterol 1993; 25 : 419-24
25) Graham DY, Malaty HM, Evans DG, Klein PD, Adam E. Epidemiology of *Helicobacter pylori* in an asymptomatic population in the United States. Effect of age, race, and socioeconomic status. Gastroenterology 1991; 100 : 1495-501
26) Lin JT, Wang JT, Wang TH, Wu MS, Lee TK, Chen CJ. *Helicobacter pylori* infection in a randomly selected population, health volunteers, and patients with gastric ulcers and gastric adenocarcinoma. A seroprevalence study in Taiwan. Scand J Gastroenterol 1993; 28 : 1067-72